REMARKS

The Office Action mailed January 25, 2006 has been carefully reviewed and the foregoing amendments are made in response thereto. Claims 1-26, 28 and 30-43 were last examined and stand rejected. Claims 8-10, 35 and 36 are canceled herein. Claims 1, 24, 31 and 37 are independent. New claims 44 and 45 have been added. Support for the new claims may be found in originally filed claims 15-19.

Applicants have amended claim 1, from which claims 2-23 depend to incorporate the limitations of claim 8, 9 and 10. As amended, claim 1 is directed at a method of amplifying a nucleic acid population from blood to obtain amplified, labeled RNA, while blocking the amplification of globin mRNA. Amplification of globin mRNA is blocked by hybridization of a reduction oligonucleotide to at least one globin mRNA in the sample, the sample is incubated with RNase H, the RNase H is inactivated, an oligo dT primer with an RNA polymerase promoter sequence is hybridized to RNA in the mixture, extended to make cDNA and the cDNA is amplified by making it double stranded and making RNA using an RNA polymerase.

Independent claim 24, from which claims 25, 26, 28, 30, 44 and 45 depend is directed at a method for analyzing nucleic acid from blood by amplifying nucleic acids in the sample while blocking amplification of globin by hybridizing a reduction oligonucleotide to globin mRNA and digesting the resulting RNA:DNA hybrid. The amplified nucleic acid is analyzed by hybridization to an array of probes.

Independent claim 31 as amended, from which claims 32-34 depend, is directed to a method for analyzing nucleic acids from blood by removing globin mRNA by forming a complex between globin mRNAs and a reduction oligonucleotide and removing the

complexes, amplifying remaining RNA using random primers and hybridizing resulting cDNA to an array. Support for the amendments to claim 31 may be found in original claims 35 and 36, which have been canceled herein.

Independent claim 37, from which claim 38-43 depend is directed at a method for analyzing nucleic acid from blood by amplifying mRNA while blocking amplification of globin mRNA, labeling the amplified sample and hybridizing the labeled sample to an array.

As amended all of the claims have the limitation that globin mRNA amplification is blocked during an RNA amplification step.

Information Disclosure Statement

The information disclosure statement submitted on July 12, 2004 contained 3 pages of form 1449. The office action mailed 1/25/2006 included copies of the first 2 pages of form 1449 but not the final page. Applicants respectfully request that the Examiner consider the references provided on the final page of the form 1449 submitted on July 12, 2004 and initial the 1449. A copy of that page is included herewith as an attachment.

Rejection of Claim 40 Under 35 U.S.C. § 112 should be withdrawn

Claim 40 stands rejected for insufficient antecedent basis for the term "blocking molecules". Claim 40 has been amended to be dependent on claim 39 which provides antecedent basis for "blocking molecules". Applicants respectfully request withdrawal of this rejection.

Rejection of Claims 37, 38 and 42 Under 35 U.S.C. § 102 should be withdrawn

In paragraph 5 of the Office Actions, claims 37-38 and 42 are rejected over Lockhart et al. (Nat Biotech, 1996, vol. 14 pp1675-1680). Applicants respectfully traverse this rejection.

Claim 37, from which claims 38 and 42 depend, is directed to a method of amplifying a nucleic acid sample from a blood sample while blocking amplification of ***globin mRNA***, labeling the sample, hybridizing the labeled sample to an array and analyzing the hybridization pattern. Lockhart does not teach blocking amplification specifically of globin mRNA as required by claim 37 and therefore fails to anticipate each and every element of the claims.

Rejections of Claims Under 35 U.S.C. § 103(a) should be withdrawn

The amendments to claim 1 obviate the 103 rejections of claims 1-7 and 11-23. In paragraph 7, claims 1, 2 and 4 are rejected over Mugasimangalam et al., (U.S. Patent 6,544,742) (hereinafter "Mugasimangalam") in view of Kempe et al., (US Patent 4,661,450) (hereinafter "Kempe"). Claim 1, from which claims 2 and 4 depend, has been amended to add the limitation that the at least one reduction oligonucleotide hybridizes to at least one globin mRNA in the sample. Neither Mugasimangalam nor Kempe teach or suggest that a target for the reduction oligonucleotide is a globin mRNA.

In paragraph 8, claims 3 and 6 are rejected over Mugasimangalam in view of Kempe and further in view of Rabin. As discussed above, claim 1 has been amended to add the limitation that the reduction oligonucleotide hybridizes to globin mRNA. Claims 3 and 6 depend from claim 1. Neither Mugasimangalam nor Kempe teach that globin

mRNA is the target of the reduction oligonucleotide and Rabin fails to remedy this deficiency.

In paragraph 9, claims 3, 5 and 7 are rejected over Mugasimangalam in view of Kempe and Stamatoyannapoulos et al (20030170689) (hereinafter "Stamatoyannapoulos"). As discussed above, claim 1 has been amended to add the limitation that the reduction oligonucleotide hybridizes to globin mRNA. Claims 3, 5 and 7 depend from claim 1. Neither Mugasimangalam nor Kempe teach that globin mRNA is the target of the reduction oligonucleotide and Stamatoyannapoulos fails to remedy this deficiency.

In paragraph 10, claims 8 and 9 were rejected over Mugasimangalam in view of Kempe and further in view of Kwoh *et al.* (US Patent 5,055,393). Claims 8 and 9 have been canceled herein.

In paragraph 11, claims 10-12 are rejected over Mugasimangalam in view of Kempe and further in view of Baker et al., (US Patent 5,643,780) (hereinafter "Baker"). The limitations of claim 10 have been added to claim 1 and claim 10 has been canceled herein. As amended, claim 1 has all the limitations of claims 8, 9 and 10. Claim 11 has been amended to be to be consistent with claim 1 and claim 12 has been amended to be dependent on claim 1. The amendments to claim 1 obviate this rejection of claims 11 and 12.

In paragraph 12, claims 13 and 31-34 are rejected over Mugasimangalam in view of Kempe, Baker and Rampersad (U.S. Patent 5,830,712) (hereinafter "Rampersad"). With regard to claims 13 and 14, this rejection is obviated by amendment of claim 1 from which claims 13 and 14 depend. With regard to claims 31-34 this rejection is obviated by

amendment of claim 31 to incorporate the limitations of claims 35 and 36. As amended the claim requires random priming of target RNA, labeling of cDNA and hybridization to an array of probes.

In paragraph 13, claims 15-19 are rejected over Mugasimangalam in view of Kempe, Baker and Adams (Nature 1995, supplement, vol. 377, p.3-17) (hereinafter "Adams"). The term "consists essentially of" is intended to be closed language. The probes have a sequence identical to the sequence provided in the sequence listing.

In paragraph 14, claims 20-23 are rejected over Mugasimangalam in view of Kempe and further in view of Augello (US Patent 6,602,718) (hereinafter "Augello"). As discussed above, claim 1 has been amended to add the limitation that the reduction oligonucleotide hybridizes to globin mRNA. Claims 14 and 20-23 depend from claim 1. Neither Mugasimangalam nor Kempe teach that globin mRNA is the target of the reduction oligonucleotide and Augello fails to remedy this deficiency.

In paragraph 15, claims 24-26, 28, 30, 31, 35 and 36 are rejected over Mugasimangalam in view of Kempe and further in view of Lockhart et al., (1996, Nature Biotech, vol. 14, pp 1675-1680). Claim 24, from which claims 25-30 depend, is directed to a method for analyzing nucleic acid from a blood sample wherein unblocked nucleic acid sequences are amplified while globin mRNA is blocked from amplification. Neither Mugasimangalam nor Kempe teach that globin mRNA is the target of the reduction oligonucleotide and the Examiner has not cited any teaching in Lockhart to remedy this deficiency.

In paragraph 16, claim 39 is rejected over Lockhart, Baker and Rampersad. Claim 39 depends from claims 38 which depends from claim 37. Claim 39 is directed at

a method for analyzing a nucleic acid sample from blood by amplifying mRNA from the sample while blocking amplification of globin mRNA, labeling the amplified sample, hybridizing the amplified sample to an array of probes and analyzing a resulting hybridization pattern. The amplification step includes a reverse transcription step primed with an oligodT primer to make cDNA which is subsequently amplified. The amplification of globin mRNA is blocked by hybridization of a blocking molecule to the globin mRNA prior to reverse transcription.

Lockhart teaches amplification of mRNA and hybridization of mRNA to an array. Rampersad is cited as disclosing a method for blocking amplification of target mRNA by hybridization of one or more blocking molecules to the target prior to reverse transcription of unblocked molecules. Rampersad does not teach that globin mRNA is the target of blocking. The Examiner cites Baker as teaching that globin mRNA is highly expressed.

This rejection is traversed as the office action fails to establish a *prima facie* case of obviousness. A *prima facie* case of obviousness requires three showings:

> First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicants' disclosure.

MPEP at §2142. To establish a *prima facie* case of obviousness, the Examiner has the initial burden to provide some suggestion of the desirability of combining the references as claimed. "Either the references must expressly or impliedly suggest the

claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). *See*, MPEP §2142. The Examiner has failed to provide such a motivation to combine the methods of array analysis taught by Lockhart with the methods of cDNA library synthesis.

The Examiner is of the opinion that one of skill in the art would have been motivated to combine the teachings of Lockhart with the teachings of Baker and Rampersad because Baker teaches that globin was abundant and abundant messages were known to interfere with construction of cDNA libraries. The Examiner has not cited any teaching or suggestion in Lockhart, Rampersad or Baker that reduction of highly expressed mRNAs would be advantageous when analyzing a nucleic acid sample by hybridization to an array. In fact, as the Examiner has noted, Lockhart teaches that the array analysis method taught in Lockhart has the benefit that it is sensitive, specific and quantitative and that it is intrinsically parallel and readily scalable to the monitoring of very large numbers of mRNAs. Lockhart teaches that each mRNA is detected independently so even mRNAs that are expressed at low levels will be detected with high sensitivity and specificity. There is no suggestion or teaching in Lockhart that would lead one of skill in the art to conclude that a highly abundant mRNA would interfere with detection of an mRNA that is less abundant.

In paragraph 17, claims 40 and 41 are rejected over Lockhart in view of Mugasimangalam, Baker and Kempe. As discussed above the Examiner has failed to provide a motivation to combine the methods of Lockhart with methods of reducing

representation of abundant messages such as globin during library construction as taught in Mugasimangalem, Baker and Kempe and has therefore failed to establish a *prima facie* case of obviousness.

Mugasimangalam is cited as disclosing methods and materials for normalizing nucleic acids for preparation of cDNA libraries, using a technique called 'prime and kill'. Kempe is cited as teaching that blood can be a good source of RNA and particularly of the globin sequence. Baker is cited as teaching that an overabundance of highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species. Baker teaches that globin mRNAs are very abundant in polyA+ RNA from certain cell types and that when making cDNA libraries it may be desirable to inactivate or degrade overabundant mRNAs.

The 'primer and kill' method taught in Mugasimangalam is used to normalize representation in the library of genes that are highly expressed relative to genes that are expressed at low levels. When generating a library of clones, the representation of any given message is approximately proportional to the abundance of the mRNA in the starting material. When a sampling method of analysis is used this biases the study in favor of abundant mRNAs and against low abundance mRNAs, because the amount of samples that can be analyzed is limited. There is no suggestion or teaching in Mugasimangalam, Kempe or Baker that the undesirable impact of high abundance messages on cDNA library construction would be applicable to array analysis.

Also in paragraph 17, claim 43 is rejected over Lockhart in view of Rampersad, Baker and Augello. As discussed above the Examiner has failed to provide a motivation to combine the methods of Lockhart with methods of reducing representation of abundant

messages, such as globin, during library construction as taught in Rampersad, Baker and Augello. Therefore, the Examiner has failed to establish a *prima facie* case of obviousness.

CONCLUSION

Having addressed all outstanding issues, Applicants respectfully request reconsideration and allowance of the case. If the Examiner feels that a telephone conference would in any way expedite the prosecution of the application, please do not hesitate to call the undersigned at (408) 731-5768.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account 01-0431.

Respectfully submitted,

July 26, 2006

/Sandra E. Wells/
Sandra E. Wells
Reg. No. 52,349

Attachments: Final Page of 1449 from 7/12/2004

Legal Department
Affymetrix, Inc.
3420 Central Expressway
Santa Clara, CA 95051
Tel: 408/731-5000
Fax: 408/731-5392